AMINO AND AMIDE NITROGEN CONTENT OF THE POTATO,

SOLANUM TUBEROSUM,

AND ITS RELATION TO A SHOOT INHIBITION PHENOMENON.

RECOMMENDED:

Bonita J. Neiland

Frank J. Wooding

David D. Murray

Donald H. Dinkel
Chairman, Advisory Committee

Stephen Chamberlain
Department Head

APPROVED:

Frederick C. Dean
Dean of the College of Biological Sciences and Renewable Resources

1 May 1973
Date

Vice President for Research and Advanced Study

8 May 1973
Date

AMINO AND AMIDE NITROGEN CONTENT OF THE POTATO, *SOLANUM TUBEROSUM*, AND ITS RELATION TO A SHOOT INHIBITION PHENOMENON.

A
THESIS

Presented to the Faculty of the

University of Alaska in Partial Fulfillment

of the Requirements

for the Degree of

MASTER OF SCIENCE

Deborah Donoghue McCown, B.S.
Fairbanks, Alaska
May, 1973

ABSTRACT

Solanum tuberosum L. plants grown in non-nutritive media exhibit a shoot inhibition in light which follows normal tuber dormancy. Shoot inhibition is expressed as retardation of leaf development. Physiological age, injury, and nitrate fertilization appeared to be factors in overcoming the inhibition. Studies included comparison of inhibited and non-inhibited shoots fertilized with nitrate and ammonium N sources and analysis of amino-N and amide-N concentrations.

Nitrate fertilization produced normal plants. Ammonium fertilized plants remained inhibited, although it was apparent that ammonium was absorbed. Estimates of concentrations of amino-N and amide-N indicated that while nitrate fertilization produced morphologically normal plants, plant nitrogen reserves were not being metabolized. High amino-N and amide-N levels were found in storage tissues of ammonium fertilized plants indicating that absorbed ammonium was being assimilated into organic compounds, but not utilized for immediate growth.

Visible ninhydrin absorption spectra of alcoholic extracts were analyzed. Each tissue showed a distinct complement of amino acids and amides and the composition was relatively unaffected by external nitrogen sources. Glutamine may play a significant role in amino-N and amide-N storage in inhibited shoots.

Possible mechanisms involved in limiting amino-N and amide-N are discussed.

ACKNOWLEDGEMENTS

I wish to thank a number of people for help and encouragement. First, my major professor, Dr. Don Dinkel for agreeing to take me on as a graduate student and obtaining the financial support that makes life so much easier.

I also wish to express my sincere thanks to Dr. Bonita Neiland who helped me from the beginning - by helping me through the red tape of admission to graduate school, by loaning me her three growth chambers for my entire term of study and for always listening to my problems and offering encouragement in return.

Most of all I want to thank my husband, Brent, for sharing his lab with me, for helping me salvage disasterous experiments, for always being available to discuss an idea or give advice and for reading every draft of this thesis. It is because of his unfailing support that this thesis has materialized and it is to him I wish to dedicate my thesis.

TABLE OF CONTENTS

TABLE OF CONTENTS (continued)

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

The potato is a major food for much of the modern world and because of this important role, it has been the object of considerable research. One peculiar facet of growth of the common potato, Solanum tuberosum L. apparently went unobserved for a long period of time. It was first reported in the literature in 1963 by Dinkel (6), who described an apparent inhibition of shoot growth in light that followed the normal tuber bud dormancy. This shoot inhibition was later shown to be related to nitrogen metabolism and although the tubers themselves contain a sufficient quantity of stored nitrogen to support at least initial growth, they have greatly reduced growth when subjected to light (7). Normal growth in the light was observed only: (1) after the application of an inorganic source of nitrogen to the roots, (2) when the tuber is partially decomposed following initial root and shoot development, or (3) after a sufficient time when the tuber reaches a certain physiological age which may be related to tuber senescence.

The objectives of this study were to provide preliminary information which would permit formulation of some testable hypotheses as to the inhibitor mechanism and the role of various nitrogen compounds in overcoming this inhibition. Consequently, the research described in this dissertation is along two complementary lines. First, to further detail and compare the physiological response of inhibited shoots with their non-inhibited counterparts and to evaluate the response of these inhibited shoots to various nitrogen sources. Secondly, to biochemically analyze leaf, stem, root, and tuber tissue to determine the forms of

stored nitrogen, their quantities in the various tissues, and their possible contribution in the shoot inhibition phenomenon.

BACKGROUND AND LITERATURE REVIEW

The common potato, Solanum tuberosum, is normally a vigorous plant, frequently attaining a height of two feet or more. Plants grown from tubers produce an adventitious, fibrous root system from the region immediately above the nodes of the underground stem or shoot. The first leaves produced on the shoot are typically simple while later leaves are pinnately compound with petiolated leaflets. The below ground portion of the stem has small scale-like leaves, and stolons form in the axils of these leaves. Tubers are formed by enlargement of the terminal bud(s) of the stolons. Hence, the tuber is morphologically a fleshy stem with buds or "eyes" in the axils of small scale-like leaves which are soon shed, leaving the characteristic ridge or leaf scar subtending the bud. Mature potato tubers have an inherent dormant period which varies in length with different cultivars. Under normal conditions, shoots are not produced until the tuber bud dormancy is broken.

The shoot inhibition phenomenon in S. tuberosum was first reported by Dinkel (6). He found that when non-dormant tubers were planted in non-nutritive medium, the sprouts would elongate until they reached the light at the surface of the media and then normal shoot growth and leaf development would continue only at an extremely slow rate. In contrast, root development was normal, demonstrating that the shoot inhibition was not a result of lack of root growth (although the actual role of roots in growth and development of plants is still unclear). Dinkel's work elucidated a number of features of the shoot inhibition phenomenon (7). First, it was not a variety specific response as all cultivars of S.

tuberosum tested exhibited the same response. Secondly, nitrogen appeared to be the limiting factor for growth since no inhibition was observed when nitrate was applied externally, regardless of the presence or absence of other macro nutrients in the media. However, plants given a complete macro nutrient complement had greater fresh weight at the termination of the experiment than plants treated with a nitrate source only. This might be expected since an average sized tuber would be assumed to contain only enough nutrients to give the plant a start but not enough to produce maximum plant vigor. However, Baker (1), working on transport pathways in dark sprouted tubers, found that over an eight week period 1250 µg potassium were translocated to the sprout. Within the parent tuber the potassium level declined from 4.2 mg/g dry weight to 3.4 mg/g dry weight over the same period. Thus it is apparent that the tuber can supply considerable amounts of nutrients to the developing shoot.

The general physiological state of the potato tuber appears to have an important influence on the resultant plant. This phenomenon has been alluded to in the literature by Rico (29) who studied the influence of size of seed tubers on production. He used two sets of tubers, one set containing whole tubers and the other set containing cut tubers of identical weights. He found that greater yields were obtained with cut tubers than whole tubers. Dinkel (personal communication) subjected tubers to high gravity fields and found that aerial shoot growth was greatly increased, even though the shoot was still entirely dependent on the parent tuber for nutrition. These findings directly support the

idea that the tuber contains sufficient reserves to support normal, non-inhibited shoot growth. They also indicate that wounding may release compounds in the tuber that were previously unavailable for growth.

The general growth of the potato plant is also related to the age of the tuber, that is, the elapsed time after harvest, and this has been indicated by many authors (16, 33). Plants grown from tubers of different ages produce different quantities of above-ground biomass and vary in the amount and time of tuber formation. Kawakami (16) has shown that there is a definite physiological stage at which tubers produce plants which will give maximum yield; tubers planted too early may produce unsatisfactory sprouting (juvenile degeneration) and if planted too late, degeneration due to decrepitude may result (senile degeneration). Generally, when tubers are first out of dormancy, they produce a single shoot and apical dominance at this stage is evident. As the tuber ages, apical dominance is decreased and two shoots are produced; this process is continued to a multi-shoot stage. The relationship between tuber age and shoot inhibition is unknown although as Dinkel pointed out (7) more and more shoot growth is produced as tubers age physiologically. Hence, the process of tuber senescence may be important in shoot inhibition.

There is little available information on the site of control of tuber dormancy and none on shoot inhibition control. Simmonds (3) attempted to determine by grafting experiments whether or not tuber dormancy is a transmittable factor similar to that seen in the short-day, long-day response of potatoes. His results failed to show any transmission and his conclusion was, "...that tuber dormancy is inherent in

the tubers and independent of the top; dormancy is, so to speak, built into the meristem that expresses it". It is interesting to speculate whether the site of control for shoot inhibition is similarly in the shoot meristem where the inhibition is expressed.

In a majority of studies on the physiological development of potatoes, plants were grown with nutrients inherent and/or added to the medium. If inorganic nitrogen does in fact overcome shoot inhibition as outlined by Dinkel (7) the shoot inhibition phenomenon would not be observed. For example, Humphries and French (14), looking at the possible interaction of gibberellic acid and nitrogen, grew their potatoes in "fertile light soil". They then treated some of their plants with either a supplemental application of $(NH_4)_2SO_4$ to the roots or with urea as a foliar spray and obtained no differences between treatments in the mean area per leaf.

Field studies on the response of potatoes to various N sources showed no significant difference in US #1 tonnage between plants fertilized with equivalent amounts of $(NH_4)_2SO_4$, NH_4NO_3, or $Ca(NO_3)_2$. Further studies comparing the effects of NH_4NO_3 and urea again showed no differences in yield, N in tubers, N removed from the soil, or N in the foliage (21). Thus, it appeared that field grown potatoes could utilize nitrate, urea or ammonium sources of nitrogen equally well.

Work on other plant genera shows only a species specific perference for one type of nitrogen source or another. No plant is known to require both ammonium and nitrate separately, but many species are reported to grow better with both ions than with either one separately (26).

Greidanus et al. (10), working on Vaccinium macrocarpon Ait. nutrition, found that cranberries were unable to utilize any form of nitrate and had a specific requirement for ammonium. Other workers (26) have reported that various cereals (barley, oats, rye and wheat), radish, and sugar beet grow best with nitrate while Chenopodium album L. seems to use only ammonium and can accumulate nitrate, which is not utilized. Hill-Cottingham (12) cited work by Grasmanis and Leeper which showed that apple trees grown in an inert medium and given ammonium as the only N source grew no better than trees given no N, although ammonium-N was absorbed.

The results of field experiments may not demonstrate the form of N utilized by the plant, for interconversion of N compounds in the soil can be rapid and complete (18):

$$NO_3 \longleftrightarrow NO_2 \longleftrightarrow ? \longleftrightarrow NH_4 \longleftrightarrow N_2$$

These processes are primarily carried out by soil bacteria, but can also occur as soil chemical reactions (26). Urea as an N source can be broken down rapidly by urease but can also undergo chemical transformation (24):

$$H_2N - \overset{\overset{O}{\|}}{C} - NH_2 + 2\,H_2O \longrightarrow (NH_4)_2CO_3$$

Ammonium carbonate is unstable in the soil and further decomposes to ammonium which is adsorbed in exchangeable form to soil colloids (4).

Dinkel (7) found that nitrate sources applied to the roots of inhibited shoots still attached to parent tubers could cause these shoots to overcome the inhibition, but he did not report on the effectiveness of other inorganic or organic forms of nitrogen.

The relationship between age of the plant and the amount and type of nitrogen source utilized has been intensively studied in rice. Young rice plants grow better with ammonium than with nitrate, but the preference is reversed in later stages of development (25). Potassium cyanide (10^{-4} M) added to a culture solution containing excised roots of rice plants inhibited the uptake of nitrate but not of ammonium or potassium (26). With cyanide the average uptake of nitrate was reduced to 38.3% of the controls, while for ammonium and potassium the corresponding values were 101% and 101.5%. The negligible changes found with the latter two ions strongly suggest a specific effect of cyanide on nitrate assimilation rather than a general effect on the metabolism of roots.

The chemical composition of tubers of S. tuberosum and the distribution of nitrogenous compounds has been studied in detail. Neuberger and Sanger (27) found that the total nitrogen content of several varieties of potatoes ranged from 1.16 to 1.95% of the dry weight. Of this total nitrogen content 37.0 to 63.7% was protein-N, 11.8 to 27.0% asparagine-N, 7.6 to 19.7% glutamine-N, 6.4 to 12.8% alpha amino acid-N, and 20.4 to 27.7% basic-N. Free amino acids were found to amount to 40 to 50% of the non-protein N. Slack (32) found that approximately 50% of the non-protein N fraction was present as the amides, glutamine and asparagine, and about 30% as nitrogenous bases. He also found 10 to 20% of the N was alpha amino acid-N.

The mechanism of nitrate breakdown and utilization in plants was once thought to be relatively well established but is again a subject

of debate. The classical scheme has been (8):

$$\underset{NO_3^-}{\text{NITRATE}} \longrightarrow \underset{NO_2^-}{\text{NITRITE}} \longrightarrow \underset{(NOH)_2}{\text{HYPONITRITE}} \longrightarrow \underset{NH_2OH}{\text{HYDROXYLAMINE}} \longrightarrow \underset{NH_4^+}{\text{AMMONIA}}$$

Recent work by Hewitt et al. (11), working on both microorganisms and higher plants, has shown that hyponitrite is very unstable at physiological pH (7.0 to 7.5) and when ^{15}N hyponitrite is injected into leaves, it is first oxidized to nitrite before reduction to $^{15}NH_4^+$. They also felt that hydroxylamine could not be an intermediate product since it is highly toxic and kinetic data exclude it as a possibility. Further, nitrite severely inhibits hydroxylamine reduction, even when hydroxylamine is present in much larger quantities. They propose that there is probably not a $1e^-$ reduction state of nitrite and that nitrite is reduced directly to ammonia. Many other researchers (8) have felt that enzymatic data support the intermediate theory. Hewitt et al. (11) propose that nitrite reductase and hydroxylamine reductase are the same enzyme with two allosteric sites.

The subject of nitrate reduction in higher plants has been adequately reviewed by Beevers and Hageman (2). Nitrate reductase is an adaptive enzyme, its activity is inducible by nitrate and molybdenum, and it appears to have a specific requirement for NADH as an electron donor. The location of the enzyme in the plant is a subject of some debate, but the preponderance of evidence suggests that the greatest nitrate reductase activity occurs in leaf tissue. Wallace and Pate (42) found that Xanthium translocates 95% of the absorbed nitrate to the shoots and only negligible amounts are metabolized in the roots. Pate (28), working on

Pisum, found that roots were able to reduce the majority of nitrate absorbed, but only if plants were maintained on a nitrate concentration below 10 ppm. At concentrations above 10 ppm, nitrate was translocated to the shoots and reduced there.

The fate of the ammonium ion, whether derived from the reduction of nitrate, from an external ammonium source, or from the breakdown of other amino acids, is generally accepted. Alpha-keto glutaric acid is the primary acceptor of ammonia and the reaction is catalyzed by L-glutamic dehydrogenase and DPN (diphosphopyridine nucleotide) coenzyme via the intermediate, alpha-imino glutaric acid (9, 5). Glutamic acid is the primary participant in transamination reactions resulting in the formation of other amino acids, thus it can act as a pivotal compound (9,31). Glutamine similarly plays an important dual role in amino acid metabolism by making available ammonium (i.e it acts as a nitrogen storehouse) and by providing a source of glutamic acid. The formation of asparagine proceeds in a manner similar to glutamine (8). Since the synthesis of an amide uses 3460 calories, the reaction requires the participation of ATP. The amide radical has a chemical character similar to the amino radical except it is relatively easier to cleave, hence the in vivo cycling of this ammonium ion occurs more frequently.

Other keto acids can act as acceptors of ammonium, for example when aspartic acid is formed from fumaric acid by aspartase (8). Aspartic acid can also be formed from oxalacetic acid, but this occurs only via a transaminase and it is thus a secondary amino acid.

In the common formation of glutamine and asparagine during seed germination, the quantity of each fluctuates according to the plant species concerned. It occasionally occurs that during the germination process of a single species, first one amide and then the other predominates. Apparently the two amides are easily interconverted (8):

$$\text{GLUTAMINE} + H_2O \underset{\text{glutamine synthetase}}{\overset{\text{glutaminase}}{\rightleftharpoons}} \text{GLUTAMIC ACID} + NH_3$$

$$\text{GLUTAMIC ACID} + \text{OXALACETIC ACID} \overset{\text{transaminase}}{\rightleftharpoons} \text{ALPHA-KETO GLUTARIC ACID} + \text{ASPARTIC ACID}$$

$$\text{ASPARTIC ACID} + NH_3 + \overset{\text{asparaginase}}{\rightleftharpoons} \text{ASPARAGINE}$$

The participating compounds and enzymes are present in every cell, thus the prerequisites for the reaction are widespread.

Steward and Thompson (37) caution that these two amino acid amides cannot be considered homologs of each other as asparagine has a ring structure while glutamine shows a chain-like configuration. Consequently their reactivities are also different.

There is no correlation between the relative proportion of free amino acids in tubers and their occurrence in the alcohol insoluble or protein fraction. Compounds such as gamma amino butyric acid occur free in the tissue (36) but do not occur in protein. Proline is much more abundant in the combined than free state. Thus it may be possible to study amino acid levels relatively independently of protein levels, at least in healthy non-growing tissues.

MATERIALS AND METHODS

Plant Material

All Solanum tuberosum plants used were the cultivar 'Kennebec'. This was one of the original cultivars used by Dinkel (6, 7) and excellent material from several seasons was readily available in Alaska. Tubers grown in the summer of 1970 were harvested in the fall and stored at approximately 5° until used in late 1971 and early 1972. Thus these tubers were at least 15 months old and will henceforth be referred to as 1970 tubers. Tubers were also available from the 1971 growing season. These tubers were stored similarly and were approximately 3 months old when used. They will be referred to as 1971 tubers. The normal tuber bud dormancy period for Kennebec is approximately 45 days, thus all tubers had sufficient time to break dormancy.

In one experiment, several other Solanum species were evaluated for the shoot inhibition phenomenon. Species used, obtained from the U. S. Department of Agriculture Potato Introduction Station at Sturgeon Bay, Wisconsin, were:

Species	Plant Introduction Number
S. acaule Bitt.	275129 275132
S. chacoense Bitt.	320285 320287
S. demissum Lindl.	365380 365391
S. medians Bitt.	210045 310994

Species	Plant Introduction Number
S. mochicense Ochoa	283114 365334
S. phureja Juz. et Buk.	320375 320381
S. stenotomum Juz. et Buk.	234013 365344

Description of Experiments

I. The first group of experiments were designed to provide more specific morphological and developmental information on the shoot inhibition phenomenon. These included the following experiments:

1. Comparison of growth characteristics of shoots produced from 1970 and 1971 tubers.
2. Comparison of growth characteristics of shoots growing from 1971 tubers and fertilized with several nitrogen sources.
3. Comparison of growth characteristics of excised inhibited 1971 shoots fertilized with two nitrogen sources. These shoots were grown in sterilized liquid cultures. Shoots were also compared with control plants from experiment 2 (shoots growing from 1971 tubers) to determine where the site of control was for the shoot inhibition phenomenon.
4. Comparison of the growth characteristics of excised, non-inhibited 1970 shoots fertilized with two different nitrogen sources.
5. Comparison of growth characteristics of several Solanum species tubers with growth characteristics of S. tuberosum (1970 and 1971 tubers).

II. The second general group of experiments included biochemical analyses designed to provide data on location and type of nitrogen storage in inhibited vs. non-inhibited shoots. These included analysis of the free amino acid pool, the amides, glutamine and asparagine, and nitrate and nitrite. S. tuberosum tissue used included:

1. Developing shoots from 1971 tubers fertilized with two nitrogen sources. Parent tuber tissue was also analyzed.
2. Plants produced from excised shoots from 1971 tubers grown in sterilized cultures supplied with two nitrogen sources.
3. Shoots and new tubers produced from 1970 tubers.

Environmental Growing Conditions

Most of the growth experiments were carried out in controlled environment chambers. Long day conditions (16 hours at approximately 3,000 f.c.) were maintained to retard tuberization of established plants. Most of the experiments were conducted at 20° day and 13° night temperatures. Two of the experiments (I.2 and I.5) had identical replicates which were grown in the greenhouse. Plants grown in the greenhouse had a minimum of 16 hours light, and night temperatures of approximately 20°. (Day temperatures were higher and variable.)

Nutrient solutions were made according to Hoagland's formulation for solutions without nitrogen (13). Nitrogen sources were weighed separately and added at a rate of 200 ppm N. These solutions were used full strength on all experiments except I.5, the various Solanum species, where it was felt that full strength Hoaglands might be too high in salt concentration. In this particular case, a one-half strength solution was used.

In experiment I.3, 1971 shoots were grown in sterilized liquid cultures (Figure 1) to determine: (1) if control of inhibition was in the shoot meristem, and (2) what effect various nitrogen sources would have on growth of excised shoots. Eight ounce Nalgene polypropylene bottles were filled with solutions containing: (1) Hoagland's solution without nitrogen - control, (2) Hoagland's solution with 200 ppm nitrate-N (as KNO_3), and (3) Hoagland's solution with 200 ppm ammonium-N (as $(NH_4)_2SO_4$). All solutions were adjusted to pH 5.2 and solutions and plastic bottles autoclaved at 15 lbs. pressure for 15 minutes. An aeration system was devised using 18 gauge disposable hypodermic needles which were attached to an air source and forced through the wall of the plastic bottles so that air bubbled into the solution. The air, supplied by small 1.5 v pumps, was first forced through a bell jar containing a saturated solution of $CuSO_4$, then through eight inches of glass wool before entering the plastic bottles. Inhibited shoots of approximately 4 cm in length were excised from tubers with a scapel and the roots dipped in a solution of filtered 5% calcium hypochlorite for 30 seconds to try to eliminate surface contamination. These roots were then submerged in the appropriate solutions; care was taken to keep the stem, especially the cut base, out of the liquid. Solutions were changed a minimum of once a week and the experiment terminated after 4 weeks.

In all other growth experiments plants were grown in arcillite, a clay mineral (10 to 40 mesh particle size range) originally manufactured as a soil amendment. Although arcillite does not contain any nitrogen,

Figure 1. Apparatus used for growing excised shoots from Solanum tubers in sterilized liquid cultures (Experiment I. 3.).

it does contain other elements available to plants. The chemical composition of arcillite is (45):

	%
Silica (SiO_2)	69.9
Alumina (Al_2O_3)	14.5
Iron (Fe_2O_3)	5.4
Lime (CaO)	0.9
Magnesium (MgO)	0.9
Phosphorous, Manganese, Copper	Trace as shown by spectrographic analysis
Volatile matter at 1110°	1.0

Arcillite was thought to be a superior medium as it does not dry out as fast as some of the other possible choices, such as perlite, nor do the roots grow into the pores of the particles as they do with perlite. Thus separation of root tissue is fast and can be achieved with a minimum of injury and loss. The surface of the medium in all pots was covered with approximately one-half inch of styrofoam beads to retard algal growth.

Tissue Sampling

Plants from the above experiments used for biochemical analysis were sampled by first washing the media from the roots and removing excess moisture with paper toweling. Then the various tissues (when present) were separated into roots, stolons, stems (the top 1 cm of the terminal was included with leaf tissue), leaves and tubers. Roots, stolons, and

stems were chopped into 1 cm pieces and a subsample of approximately 5 g taken. Leaves were sampled by taking a 5 g portion of whole leaves (petioles included). Two different types of tuber samples were taken: (1) the outer portion including the "skin", cambium, and vascular bundle; or (2) the pith. Tuber samples were 8 to 10 g each. All samples were placed in screw-capped vials with 80% ethanol and stored at -30° until extraction.

Evaluation of Possible Extraction Methods

An effective approach to differentiating the forms of nitrogen within a tissue, a major objective of this study, involves differentiating protein and non-protein components. This is especially important for potato tissue since amides represent an important storage form of nitrogen (33) and, unfortunately, the methodology of amide determination requires that these fractions be physically separated (41).

Bell (3) did a critical study of methods for separating protein from the non-protein nitrogen fraction and investigated the use of heavy metal precipitation, heat, trichloroacetic acid, ethanol (various percentages) and a phenol : acetic acid : water mixture. Her conclusion, ironically, was that the results, "...demonstrate convincingly the dependence of NPN (non-protein nitrogen) values on the method by which they are determined, and the lack of any significant relation between the results obtained by any one method on a range of materials, or by a range of methods on one material".

Since the majority of researchers interested in amino acid metabolism have utilized ethanol extracts, their methods were explored for

adaptability in this study. However, amides have limited solubility in ethanol (43):

Compound	Solubility grams/100 ml water, 25°	Absolute enthanol, 25°
L-glutamine	4.25	0.00046
L-asparagine	2.46	0.0003
L-glutamic acid	0.89	0.037
D,L-aspartic acid	0.82	0.032

It is important to note that the above solubilities are all in 100% ethanol and could be quite different in a lower percentage solution. Further, in plants the amino acids and amides will be present as the more soluble salts, not as pure acids. Steward and Street (35), working with the amides of potato tubers, attempted to overcome this problem by utilizing a hot water extract. However, the use of ethanol, a superior protein precipitant (41), seemed preferable and thus a number of preliminary experiments were conducted to find if it could be utilized as an extractant for amides in potato tissues.

Samples (50 mg) of asparagine-HCl were placed in test tubes and 10 ml of 20%, 40%, 60%, or 80% ethanol added to the tubes. Solutions were kept at room temperature and mixed occasionally for 15 minutes. Visual observations were made throughout the period. Although the amide was solubilized almost immediately in the 20% solution, at the end of the 15 minute period no amide crystals could be detected in the 80% solution either. Since potato tuber tissue would normally be expected to have no

more than 10 mg amide/g dry weight (27), these solubilities (as indicated by asparagine-HCl) seemed adequate to permit an ethanol extraction.

As a final check, a sample simulating plant tissue was constructed using 100 mg asparagine (anhydrous, not the salt), 500 mg cellulose, and 125 mg starch. These compounds were placed in centrifuge tubes and 25 ml of 80% ethanol added. The tubes were shaken vigorously and allowed to stand 15 minutes at room temperature, and then centrifuged for 15 minutes at 2500 x g. The supernatant was decanted and the insolubles reextracted two times with 25 ml of ethanol solution. Each extract was analyzed separately for amino acid concentration (46). Results were plotted on a standard curve also prepared with asparagine. In the first extract, 11.7 mg were recovered, in the second 10.8 mg, and in the third extract 6.2 mg. Thus, a total of 28.7 mg of amide or approximately 36 mg/100 ml were extracted. Since 10 g fresh weight (2 g dry weight) of tissue is conveniently extracted in 100 mls, these solubilities are significantly higher than the expected maximum physiological levels of 20 mg (20 mg/100 ml extractant).

The methodology using ethanol extractions has varied according to the researcher, tissue, etc. (19, 20, 22, 39, 44). Kliewer (19), working with grapes, extracted organic acids and sugars with a 95% ethanol solution, simmered the mixture for 1 hour on a steam bath, and followed this by two 30 minute similar reextractions. On the other hand, Kocher and Valenzuela (20) extracted blueberry tissue for 20 hours at 5° with 80% ethanol. This was repeated three additional times although the time period was shortened to 1 hour. Considering the diversity of these

methods, a preliminary experiment was conducted on potato to determine which method kept protein contamination to a minimum, while effectively extracting amino acids and amides.

Ethanol (at 80% concentration) is an effective protein precipitant and thus was utilized for the first extraction. However, after this initial precipitation, the possibility of utilizing lower concentrations of ethanol to increase the solubilization of amides in subsequent extracts was investigated. Samples were macerated in a Sorval Omni-Mix for 1 minute and allowed to stand for 15 minutes in either an ice bath or a boiling water bath before centrifuging and reextracting. Determinations for free amino acids (46) and protein (23) were done on each sample (Table 1). The results show that nearly identical amounts of free amino acid were retrieved from either hot or cold extracts. In the second extraction a series of extracts ranging from 20 to 80% ethanol were compared. Although there was an increase of 0.52 mg amino acid (approximately 5% of the first extract), when the sample was reextracted with 20% ethanol there was also an 8.9 mg increase in protein (approximately 15% of the first extract). Thus it was concluded that the higher concentration of ethanol (80%) was preferable for all extractions and for convenience room temperatures could be utilized.

Extraction and Analytical Methodology

On the basis of all of the above information on separation of the non-protein nitrogen fraction in plants, a single extraction procedure was devised and used for all samples. The solvent used was 80% ethanol and the maximum fresh weight of tissue extracted with 1 ml of solvent

Table 1. A comparison of the effectiveness of free amino acid extraction from potato tuber tissue utilizing various percentages of ethanol at two temperatures (Hot, 75°; Cold, 5°).

	1st Extraction			2nd Extraction			3rd Extraction		
		Recovery			Recovery			Recovery	
Tissue Weight (gm)	Extract % EtOH. Temp.	mg Amino Acid	mg Protein	Extract % EtOH. Temp.	mg Amino Acid	mg Protein	Extract % EtOH. Temp.	mg Amino Acid	mg Protein
5	Hot-80%	4.8	----	Hot-80%	0.09	----	Hot-80%	0.016	----
		5.0	----		0.09	----		0.011	----
		5.1	----		0.08	----		0.030	----
10	Hot-80%	9.5	----	Hot-80%	0.20	----	Hot-80%	0.08	----
		9.7	----		0.21	----		0.07	----
		10.2	----		0.28	----		0.06	----
		10.5	75.6		0.28	0.8		0.06	1.0
		11.1	74.6	Hot-20%	0.45	2.8		----	----
10	Cold-80%	11.4	----	Cold-80%	0.16	----	Hot-20%	0.30	5.8
		11.2	----		0.19	----		0.25	6.1
		11.2	----		0.22	----	Cold-20%	0.20	4.5
		10.0	57.8		0.34	0.8		0.19	7.4
		10.3	60.7		0.17	1.6		----	----
		9.4	63.0	Cold-70%	0.24	2.8		----	----
		9.4	61.7	Cold-60%	0.27	4.9		----	----
		9.6	65.5	Cold-50%	0.31	5.9		----	----
		9.8	60.7	Cold-20%	0.69	10.5		----	----

was 0.1 gm. Samples were macerated in approximately 35 ml of ethanol with a Sorvall Omni-Mix for 1 minute with the grinding chamber kept in an ice bath to reduce unnecessary heating and foaming of the sample. The liquid was then poured into 100 ml graduated cylinders and the solution brought to a final volume of 100 ml. The cylinders were placed on a magnetic stir plate and the liquid column mixed to evenly disperse all solid material. As the sample mixed, four 25 ml aliquots were removed with a 25 ml volumetric pipet, three of the aliquots were transferred to preweighed pans for dry weight determination and the remaining aliquot centrifuged for 15 minutes at 2500 x g. The supernatant from this sample was saved for analysis and the insoluble material reextracted with two successive 25 ml portions of 80% ethanol (room temperature). The supernatants were bulked, thoroughly mixed, and subsampled. These samples were stored at -30° until analyzed.

Bell (28) discusses two possible sources of error which may be of concern in extraction of the non-protein nitrogen fraction, namely hydrolysis of nitrogen-containing polymers, and adsorption of non-protein nitrogen compounds into protein. Her data show that hydrolysis occurred in significant quantities only in the acid precipitation methods. On the problem of coprecipitation, her conclusion was that it did occur to a certain extent but that it would not significantly effect the determination of non-protein nitrogen. Although determination of the quantity of non-protein nitrogen coprecipitated with protein or other alcohol insoluble compounds was beyond the scope of this research, the multiple extraction routinely used in this study insured adequate

washing of the precipitate and subsequent recovery of the non-protein components.

The biochemical analyses all utilized standard procedures, essentially unmodified, and included the total free amino acids described by Yemm and Cocking (46) and the ammonium, amide, and nitrate-nitrite analysis of Varner et al (41).

RESULTS

Morphological and Developmental Observations

Experiment I. 1. Comparison of growth characteristics of shoots produced from 1970 and 1971 tubers.

Marked differences in shoot growth were produced from 1970 and 1971 tubers (Figure 2). Shoots produced by 1971 tubers were inhibited, little shoot elongation occurred, and leaf development was minimal. In contrast, more shoots were produced by 1970 tubers, the average shoot height was greater, and leaves were normally expanded and held at right angles to the stem. The 1970 tubers showed no pathological decomposition and were similar in appearance to the 1971 tubers. When the experiment was terminated, interiors of these tubers were examined and found to have a normal appearance, visually resembling younger tubers. It is unlikely that shoots produced by 1970 tubers would ever develop into vigorous plants because of the degree of senility of the tuber. Also it is apparent that tubers more than 15 months old were capable of producing non-inhibited shoots. Hence, physiological age of the tuber appears to be directly related to the degree of shoot inhibition.

Another interesting feature was illustrated by the 1970 tubers used in this experiment; it was impossible to differentiate between shoots produced by tubers that were undergoing pathological decomposition and those tubers that were still intact without examining the parent tubers themselves. This implies that the shoots had approximately the same nutrient supply and is further evidence that although the tubers

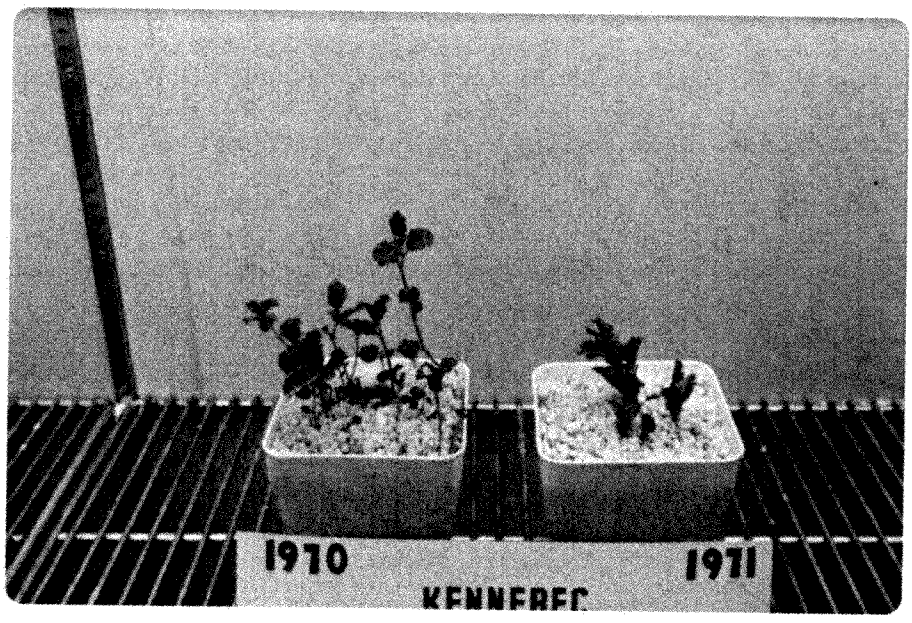


Figure 2. Comparison of growth characteristics of shoots produced from 1970 and 1971 tubers that were grown in non-nutritive media.

contain sufficient nutrients for shoot growth, these nutrients are not always available to support shoot growth.

Although the majority of 1970 tubers planted were subsequently attacked and degraded by pathologic organisms, about 20% were not affected. Of these remaining plants, approximately 50% never produced shoots, but translocated material from the parent tuber through stolons to newly developing tubers. Thus vegetative reproduction was continued without production of the typical aerial plant stage. It is apparent that these tubers had senesced to such a degree that they were incapable of producing normal shoots and roots. The normal growth pattern however, probably could have begun again once these new tubers had broken normal tuber dormancy.

Experiment I. 2. Comparison of shoots produced from 1971 tubers fertilized with several nitrogen sources.

This experiment provided some interesting physiological information about the shoot inhibition phenomenon and about the effect of various nitrogen sources. Figure 3 shows the response of shoots growing from 1971 tubers to ammonium, nitrate and urea nitrogen sources. It is obvious that the nitrate source produced the most significant effect as many large, expanded, compound leaves were produced. A similar response was observed by Dinkel (2). Response to the ammonium-N and urea-N treatments was more intermediate than that of NO_3. While shoots had unquestionably elongated when compared with control plants, leaf development was not equivalent to that produced by the nitrate treated plants (Figure 4). Leaves on the ammonium and urea treated plants had

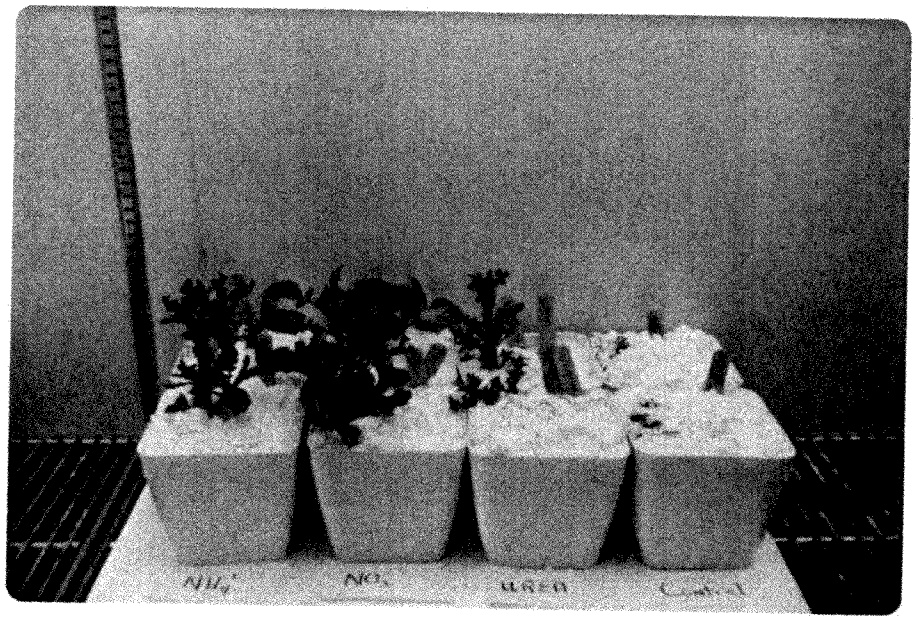


Figure 3. Comparison of growth characteristics of shoots growing from 1971 tubers fertilized with several nitrogen sources. Plants photographed 21 days after fertilization.

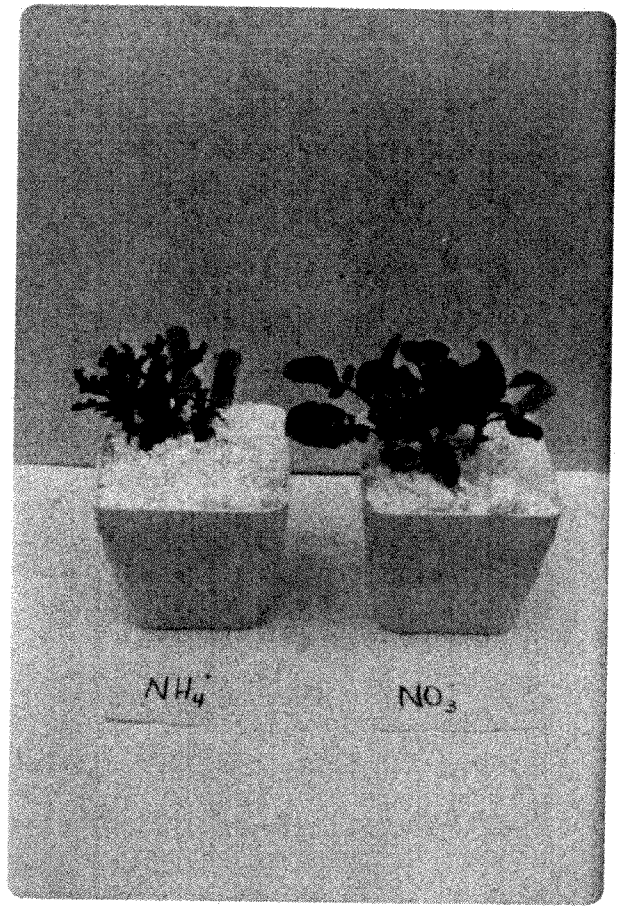
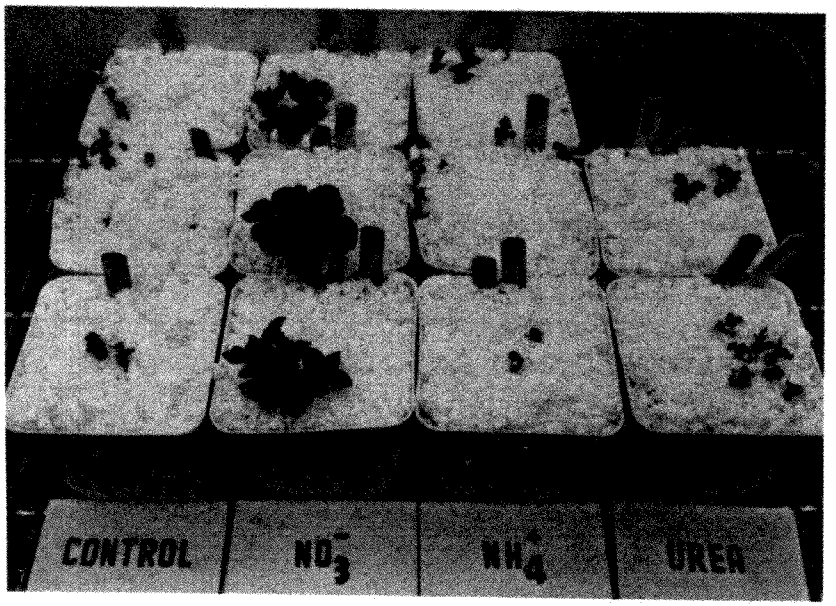


Figure 4. Comparison of growth characteristics of ammonium and nitrate fertilized shoots growing from 1971 tubers. Plants photographed 21 days after fertilization.

fewer leaflets and almost no petioles. Further, the leaves were curled with an acute angle between the stem and leaf blade.

The same experiment was repeated (approximately 30 days later) and again the response to nitrate was similar (Figure 5, photographs 1 to 3). Response of plants to ammonium and urea differed from the earlier experiment. As can be seen in photograph 1, plants appear to be showing the same response as before, however in photograph 2 the urea plant in the front row resembles the nitrate treated plants one week previously. This development is also present in photograph 3 where an ammonium treated plant is responding similarly. The unusual feature of these plants is that not all replicate plants showed the same response (one replicate is missing in photograph 3 as it had been harvested for analysis). Out of a total of five ammonium fertilized plants from the two experiments, four plants more closely resembled the control plants while one plant was morphologically similar to nitrate treated plants. The same response was observed in the urea treated plants; three were similar to controls and one was similar to the nitrate fertilized plants.

Table 2, part a, shows the average fresh weights for tissues of control, ammonium, and nitrate treated plants. Part b gives dry weight for the same plants and part c includes fresh weights for tissues of plants grown in the greenhouse. The largest plants were produced with the nitrate treatment while the ammonium treatment produced plants of intermediate weight. It appears that although these potato plants can absorb ammonia, they are unable to use it as efficiently as the nitrate sources. This effect has been demonstrated in other plants, but in many



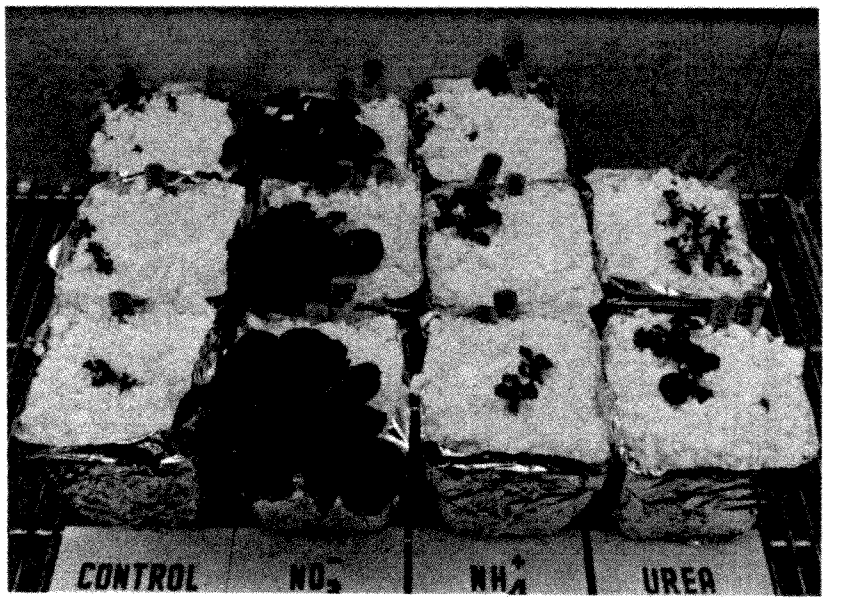


Figure 5. Comparison of growth of inhibited shoots fertilized with several nitrogen sources. Photograph 1 taken 7 days after initial fertilization, photograph 2 after 14 days, and photograph 3 after 35 days.

Table 2. The growth of Solanum tuberosum shoots produced from tubers fertilized with several nitrogen sources and grown under two environmental conditions.

Treatment	Wt. of Leaves	% Increase over Control	Wt. of Stems	% Increase over Control	Wt. of Roots	% Increase over Control	Wt. of total Plant	% Increase over Control
a. Fresh weights for chamber grown plants								
Control	5.4	---	14.9	---	12.4	---	42.5	---
NH_4	11.0	100	24.7	56	17.0	37	55.0	29
NO_3	34.5	540	34.9	134	35.3	185	108.6	132
b. Dry weights for chamber grown plants								
Control	0.7	---	1.5	---	0.7	---	2.9	---
NH_4	1.9	172	3.6	140	2.1	200	8.7	155
NO_3	4.1	486	4.7	313	2.6	272	11.4	293
c. Fresh weights for greenhouse grown plants								
Control	3.6	---	19.1	---	4.7	---	31.6	---
NH_4	6.5	81	21.3	11	11.4	143	50.7	60
NO_3	13.7	281	37.9	99	16.1	243	72.6	130
Average % increase over controls								
NH_4		118		72		127		85
NO_3		435		149		233		185

cases the response was attributed to a shift in medium pH (26). The pH of media in these experiments were checked at the termination and differed by no more than a 0.5 pH unit from the original pH or from the other treatments.

The data also show that leaf development in the nitrate treatments is proportionately much higher than average total plant weight increases (435 vs. 185), while the difference in per cent increases was not as great for the ammonium treatment (118 vs. 85).

Experiment I. 3. Comparison of growth characteristics of excised inhibited shoots grown in sterilized solutions containing two nitrogen sources.

The objectives in this experiment were twofold. One of these objectives was to determine if excised shoots showed the same response to various nitrogen sources as did shoots still attached to the parent tuber. The responses exhibited are shown in Figure 6 where again it can be seen that the shoots fertilized with nitrate were larger. Since the control shoots had available only the nitrogen reserves that were already present when the shoots were excised from the parent tubers, it would be expected that the nitrate treated plants would be larger. The response of ammonium treated plants differed from that of plants grown in nitrate solutions. This could be due in part to the pH of the ammonium solutions which became more acid as ions were selectively removed from the media, despite efforts to change solutions frequently enough to avoid this situation.

The second objective was to determine if the site of control for shoot inhibition was in the shoot meristem where the inhibition was

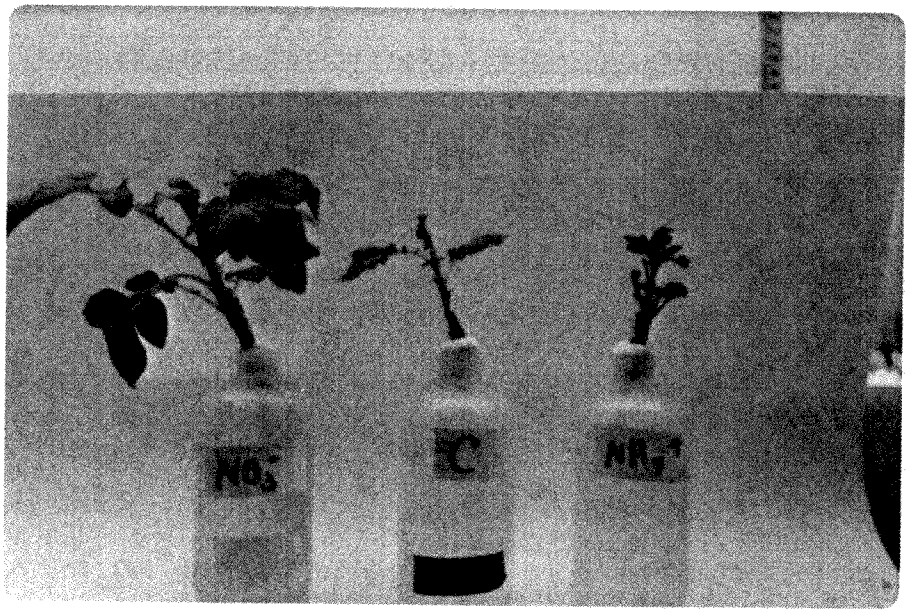


Figure 6. Growth characteristics of excised shoots grown in sterilized cultures containing two nitrogen sources. Note the leaf development of the control shoot as compared to that in Figure 5.

expressed. Measurements for average leaf length and number for the different treatments are shown in Table 3. Although control plants had fewer leaves, the leaves produced were fully expanded and averaged only 26% shorter than leaves of nitrate treated plants. The reduced number of leaves, leaf length and leaf simplicity of these control shoots were probably functions of the low level of nitrogen available.

Experiment I. 4. Comparison of growth characteristics of excised, non-inhibited 1970 shoots fertilized with two nitrogen sources.

The results from the previous three experiments indicated that ammonium was not as effective a nitrogen source as was nitrate, at least with inhibited shoots. The question was posed whether or not similar differences in growth would be observed with non-inhibited shoots. Hence, excised 1970 non-inhibited shoots were evaluated for their ability to use ammonium and nitrate nitrogen sources. The results are presented in Table 4. It is apparent that non-inhibited shoots were not able to utilize ammonium effectively and growth in height and fresh weight were only slightly greater than that of control shoots. In contrast, the growth of nitrate fertilized plants, both in height and fresh weight, was five times that of the controls.

Experiment I. 5. Comparison of growth characteristics of several Solanum species.

Although seven different species of tubers were planted, only six produced shoots. S. mochicense apparently has an exceptionally long tuber dormancy and shoots were never produced. Of the remaining six species, all produced normal shoots with none of the typical "inhibited"

Table 3. Leaf and total plant growth of excised 1971 shoots grown in sterilized liquid cultures containing two nitrogen sources.

Treatment	Average FW/Plant (g)	Avg. Leaf Length (cm)	Avg. No. Leaves/ Plant	Total Leaf Length (1 x no)
Control	6.82	6.3	2	12.6
NH_4^+	3.77	3.8	4	15.2
NO_3^-	15.00	8.8	8	70.4

Table 4. Growth of excised shoots derived from 1970 tubers (greater than 15 months old). Shoots were grown in arcillite with two nitrogen sources.

Treatment & Replicate #	Height of Shoots (cm) Time=0 (Days)	+3	+17	+24	+42	Net Increase in Height T_o-T_{24}	Net Increase in Height T_o-T_{42}
Control							
1	11.0	12.5	13.5	14.0	13.0	3.0	2.0
2	6.5	9.0	9.0	10.0	9.4	3.5	2.9
3	14.5	15.0	15.2	15.2	15.4	3.2	3.4
					$\bar{x}$ =	3.2	2.8
Mean fresh weight/plant at T_{42} = 1.0474							
NO_3^-							
1	5.5	7.0	9.5	14.3	22.5	8.8	17.0
2	7.0	9.5	13.7	19.5	27.8	12.5	20.8
3	3.0	5.5	7.5	11.9	17.8	7.9	14.8
4	8.5	10.0	12.5	19.5	29.0	11.0	20.5
					$\bar{x}$ =	9.2	15.3
Mean fresh weight/plant at T_{42} = 5.1192							
NH_4^+							
1	9.0	11.0	11.5	12.0	13.0	3.0	4.0
2	11.0	14.0	14.4	14.7	15.5	3.7	4.5
3	8.0	9.5	10.0	10.1	----	2.1	----
4	17.5	20.0	20.5	20.5	----	3.0	----
					$\bar{x}$ =	2.9	4.3
Mean fresh weight/plant at T_{42} = 2.2516*							

*Fresh weight measurement is mean of first two ammonia replicates only.

characteristics. Thus it would appear that shoot inhibition is a phenomenon confined to members of S. tuberosum.

Biochemical Analyses

Experiment II. 1. Analysis of shoots and attached tubers fertilized with two nitrogen sources.

The plants shown in Figure 5 were analyzed for total amino, amide, and inorganic nitrogen content. Figure 7 shows the concentration of amino nitrogen in mg/g dry weight in the tissues of plants fertilized with two nitrogen sources. Amino-N accumulated in stem tissue with all three treatments. Figure 8 gives the total amino-N per plant tissue and, again, values for stem tissue are higher than those of other tissues receiving the corresponding fertilization. This does not seem unique as stem parenchyma is an ideal storage site for organic forms of nitrogen, or even for nitrate as indicated by Pate (28). It is apparent, however, that leaves do not function in amino-N storage, as plants tend to maintain a constant concentrations; total tissue amounts vary directly with and as a function of increased tissue weight (Fig. 8).

Leaf tissue was the only tissue that showed a constant amino-N concentration regardless of external nitrogen fertilization. It is interesting to note that the amino-N values in tissues of all ammonium fertilized plants are the same as or higher than the corresponding values for control and nitrate treated plants. This was apparent on a concentration basis and also when total plant growth was considered. This also might be expected as ammonium (either from the ammonium source directly or from reduction of nitrate) is typically linked

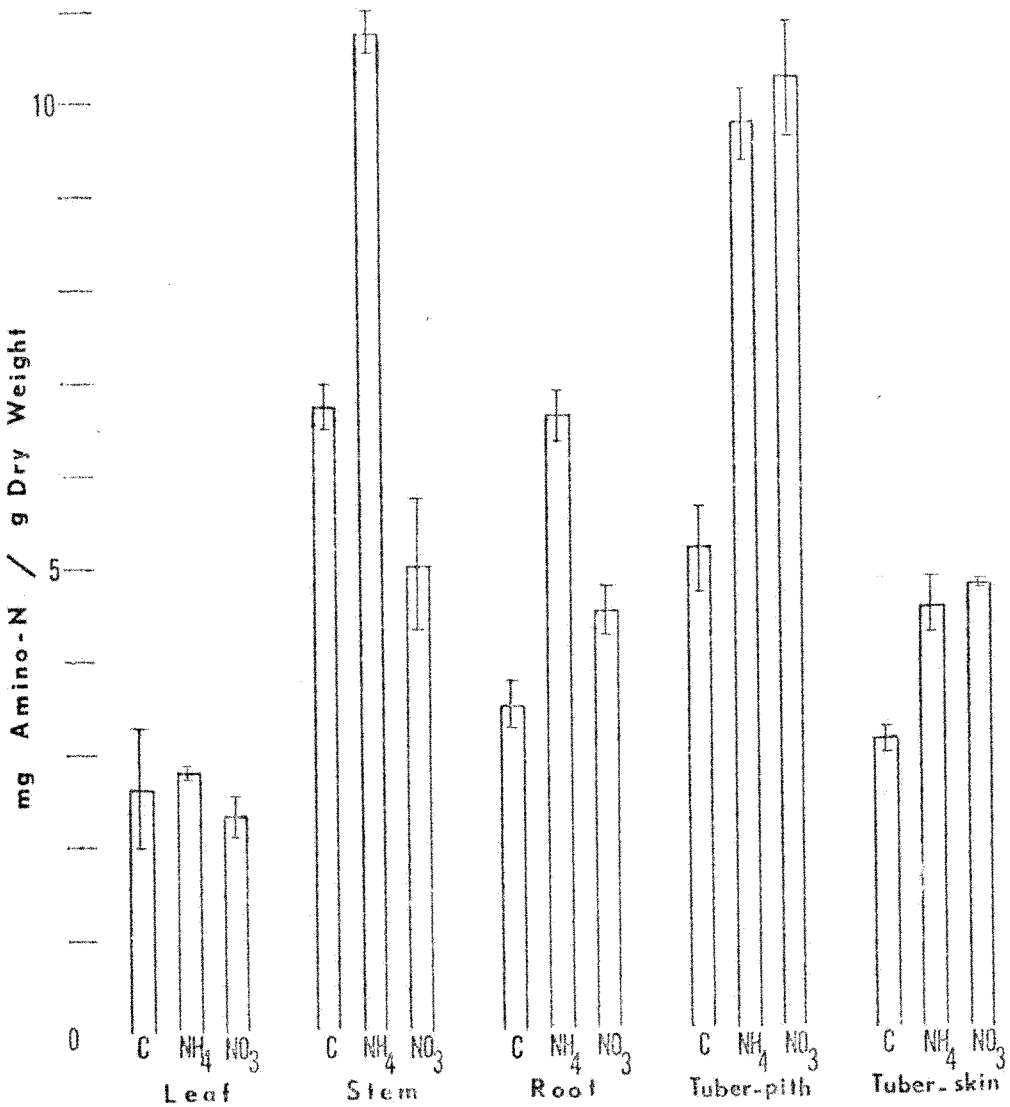


Figure 7. Concentrations of amino-N in the tissues of sprouting 1971 tubers of Solanum tuberosum fertilized with two nitrogen sources. Data presented with ± standard error of the mean.

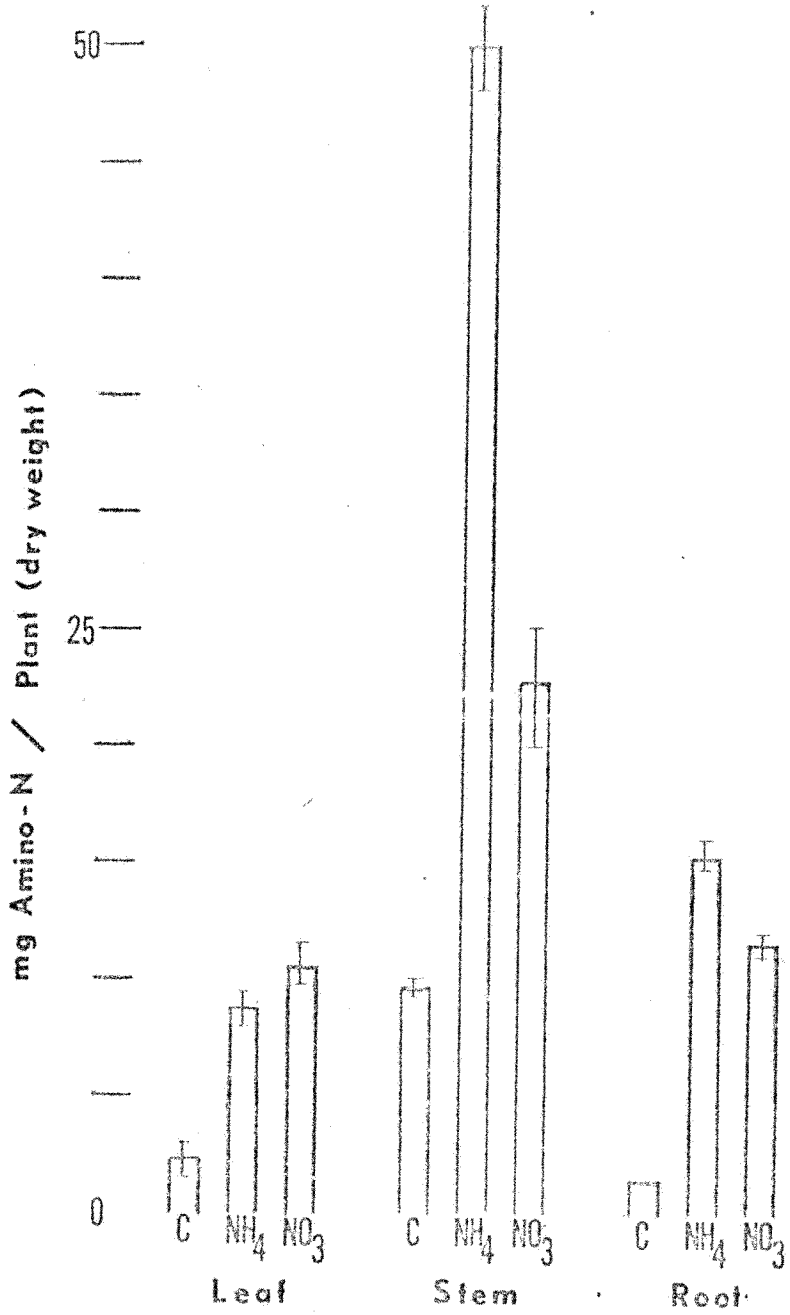


Figure 8. Total amino-N in Solanum tuberosum tissues fertilized with two nitrogen sources. Data presented with ± standard error of the mean.

to a carbon skeleton as an amino group (31) as a detoxification mechanism.

The concentration of amino-N in tuber pith indicates that an external nitrogen source results in an accumulation of amino-N. There was not as large an accumulation in tuber skin tissues compared with pith tissues but, as Smith (33) indicates, the primary nitrogen storage area of tubers is pith tissue.

In non-tuber tissues, the concentration of amino-N in control and nitrate treated plants tended to be in the same range. However, again when compared on a total tissue basis, the nitrate treated plants had higher levels due to increased growth.

Some amides (e.g. glutamine) also have a free amino (i.e. alpha) amino group and will give a positive reaction with ninhydrin (46) while other amides apparently will react differently, for example asparagine (37). The amount of amide nitrogen was measured by distillation (41). This method is specific for amide-N and does not effect amino groups. The concentrations of amide nitrogen present in the tissues are presented in Figure 9. The relative amounts of amide-N in all tissues except leaf are very similar to amino-N levels. It is evident that fertilization decreased the amount of amide-N in leaves. When the leaf amide-N data are expressed on a total tissue per plant basis the results are again similar to that seen in Figure 8 with amino-N. The amide-N concentration in control tissues was generally higher in relation to the fertilized plants than expressed by the amino-N data. Thus, it appears that the control plants have greater relative amounts of amides than plants given

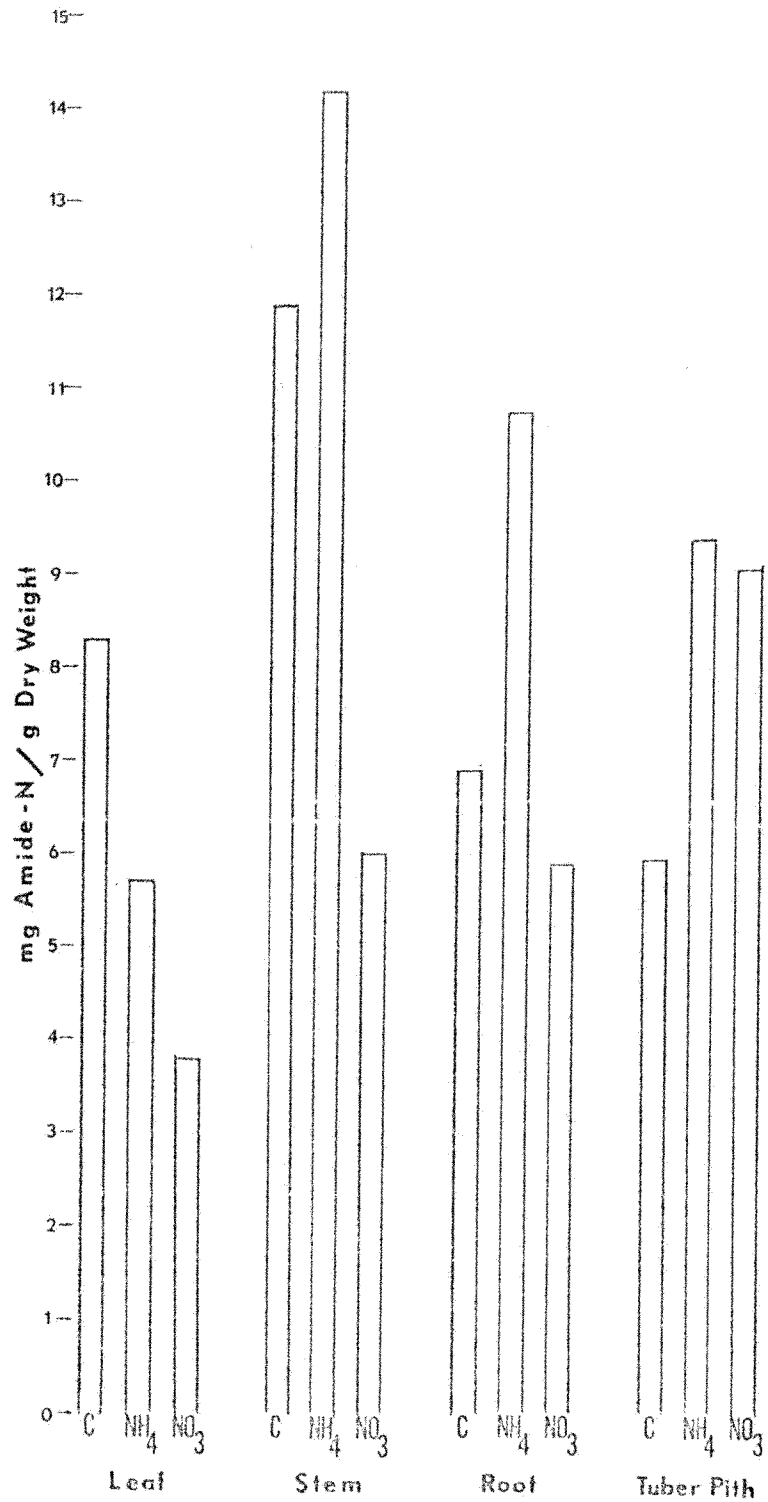


Figure 9. Concentrations of amide-N in the tissues of Solanum tuberosum fertilized with two nitrogen sources.

an external nitrogen source. All tissues show this feature but it is most obvious in stems. The ammonium treated plant also shows considerable amounts of amide-N, particularly in stem tissue.

Figure 10 presents the concentrations of amide and nitrate nitrogen in stem and leaf tissue fertilized with two nitrogen sources. Although the quantity of free ammonium present in the tissues was also measured, only trace amounts were detected under any treatment. This indicates two features: first, toxicity as a result of free ammonium in the tissue is not occurring and, second, there appears to be an effective mechanism for assimilating ammonium into organic compounds. Amide levels were higher in tissues of control and ammonium treated plants than in nitrate treated plants, and nitrate fertilized plants tended to accumulate nitrate especially in the leaves. Nitrate accumulation in plants given high nitrate fertilization is commonly reported although the highest concentrations of nitrate are generally found in stem and root tissue, not in leaf tissue (28).

Only two amides, glutamine and asparagine, are known to occur in plants in any appreciable quantity (38) and they have been shown to be major constituents of the soluble nitrogen fraction of potatoes (27). Glutamine is a straight chain amino acid amide and will react with ninhydrin to give the standard blue color. Asparagine, however, does not react with ninhydrin in the typical manner (37), but produces a brownish color. This is apparently due to the ring configuration of asparagine, and the inclusion of the amino group in the ring. Unfortunately, since glutamine can react as a typical amino acid and asparagine

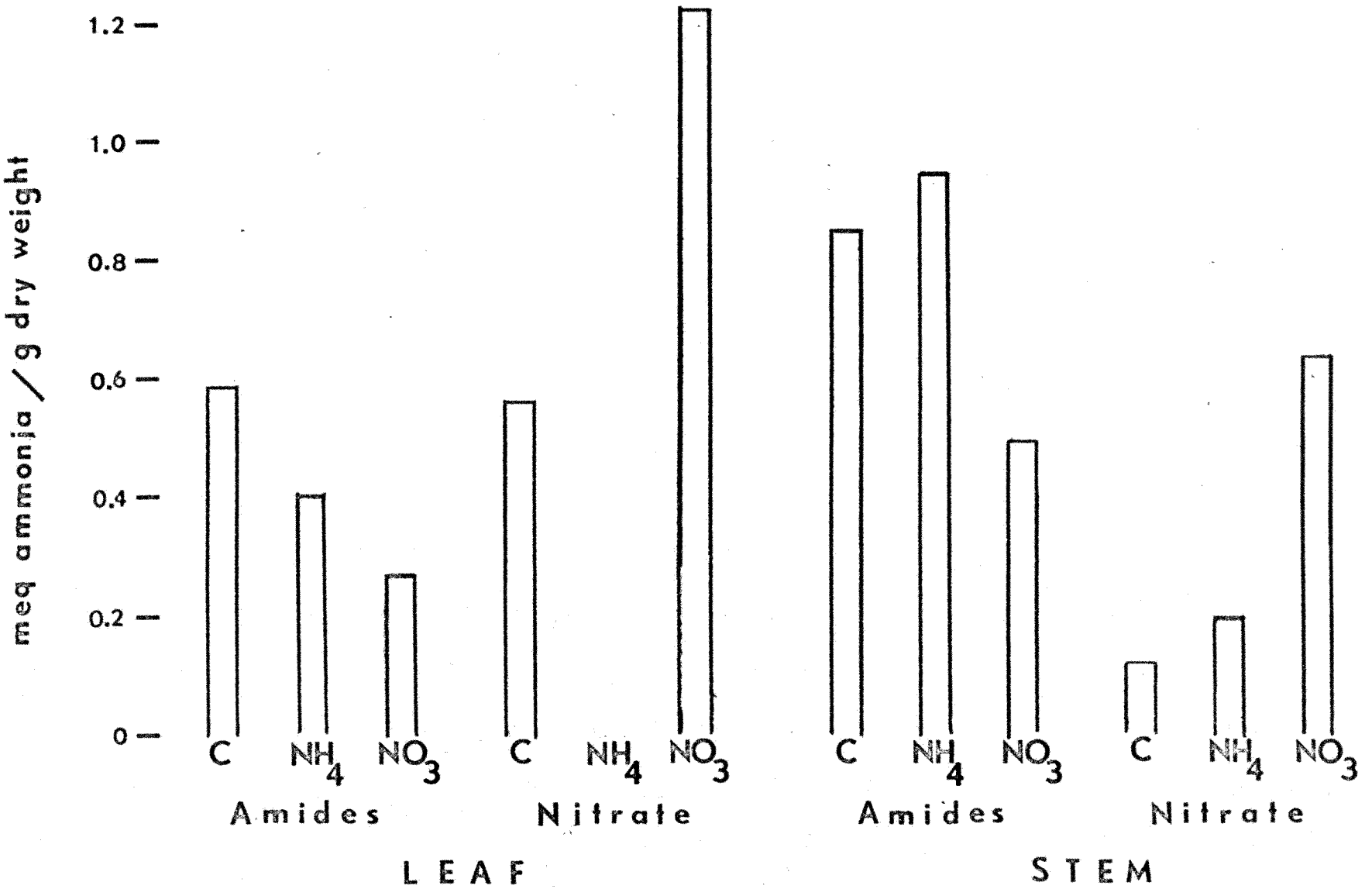


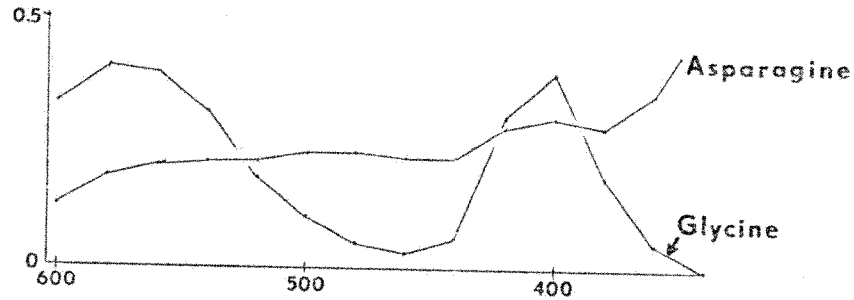
Figure 10. Concentrations of amide and nitrate nitrogen in leaf and stem tissues of Solanum tuberosum fertilized with two nitrogen sources.

cannot, evaluation of amino-N and amide-N is difficult because the contribution of each amide is unknown. However, since each one produces a different color they will have different absorption maxima. Glutamine will react similarly to glycine, which is commonly used as a reference standard for ninhydrin analysis, and will normally be read at 570 mu while Steward (37) reports asparagine absorbing at 360 and 318 mu. To help determine if the increase in amides was due to glutamine or asparagine, extracts were reacted with ninhydrin. A scan of their visible absorption spectra was taken and compared with those of glycine and asparagine (Figure 11). The results clearly indicate that each tissue has a distinct complement of amino acids and amides and the composition is relatively uneffected by external nitrogen sources. Leaf and root tissues show similar patterns while stem and tuber, the storage tissues, show a different pattern. It appears that asparagine is not the primary contributor to the high levels of amino and amide-N found in stem tissue. A visual observation of the color of the reactant solutions supported this: in all treatments leaf and root samples produced a peach color while tuber and stem of ammonium and control treatments and tuber of nitrate treatments produced a blue color. The only exception was the nitrate-stem sample where an intermediate color was produced.

Experiment II. 2. Analysis of shoots produced by 1971 tubers grown in liquid culture.

The data in Table 5 show: (a) the average concentration of amino-N in tissues from shoots attached to parent tubers, (b) the average concentration of amino-N in excised inhibited shoots grown in liquid culture

Figure 11. Comparison of spectra of ninhydrin-reacting compounds in the ethanol-soluble fractions from four plant parts of Solanum tuberosum under three nutrient regimes. Absorbance adjusted for blank absorbance and tissue weights used for extraction. Standard amide (asparagine) and amino acid (glycine) concentrations at 1.5 µg/ml.


0.5
0
600
500
400
Asparagine
Glycine

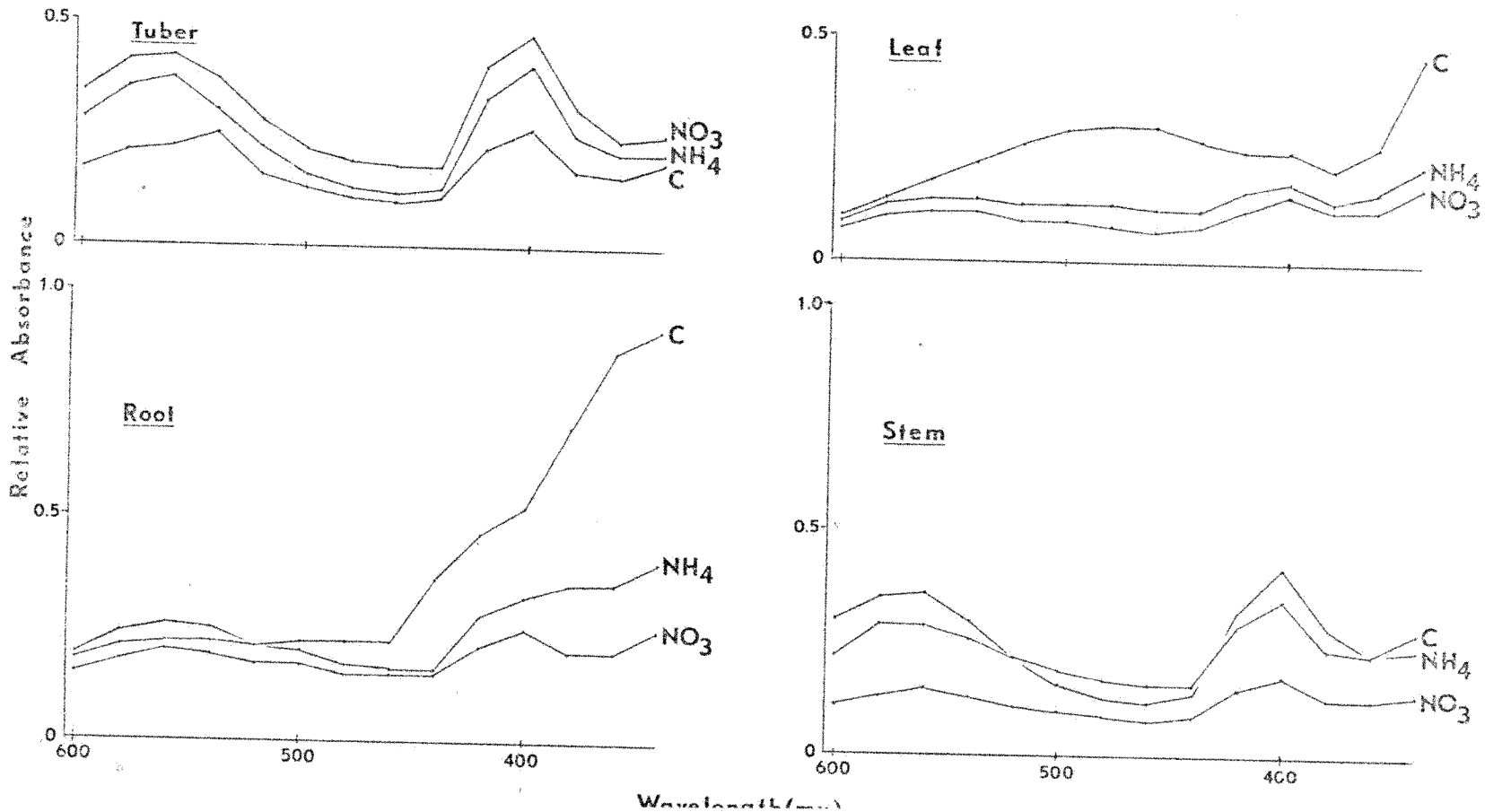

Tuber
0.5
0
NO3
NH4
C
Leaf
0.5
0
C
NH4
NO3
Root
1.0
0.5
0
600
500
400
C
NH4
NO3
Stem
1.0
0.5
0
600
500
400
C
NH4
NO3
Relative Absorbance
Wavelength (mμ)

Table 5. Amino-N concentrations in potato tissues (average mg amino-N/g dry weight)

a. Shoots attached to tubers fertilized with two nitrogen sources (Experiment II. 1).

Treatment	Leaf	Stem	Root	Pith	Skin
Control	2.64	6.72	3.46	5.18	3.10
NH_4^+	2.79	10.73	6.60	9.73	4.52
NO_3^-	2.38	4.99	4.47	10.25	4.74

b. Shoots grown in liquid cultures with two nitrogen sources (Experiment II. 2).

Treatment	Leaf	Stem	Root
Control	2.80	2.47	2.56
NH_4^+	9.89	7.78	15.61
NO_3^-	3.83	4.48	5.63

c. Shoots and new tubers produced from 1970 parent tubers (Experiment II. 3).

Treatment	Leaf & Stem	Root	New Tubers
Control	4.14	7.18	7.98

with two nitrogen sources (see Figure 5), and (c) the average concentration of amino-N in shoots and new tubers produced from 1970 parent tubers. In the excised shoots grown in liquid culture there were originally five shoots in each fertilizer treatment but only four control, three nitrate and one ammonium plant survived. The high mortality rate in the ammonium treatment and the high concentration of amino-N in all tissues, but especially roots, indicate that plants were suffering from ammonium toxicity (15). Hence no conclusions can be drawn about that treatment. In nitrate fertilized plants it is apparent that levels are the same as those found in tissues of attached shoots given similar nitrogen fertilization. The levels found in control shoots, especially the relatively low concentration in stem tissue, indicate that there was some utilization of stored reserves. This may be a function of several factors and these will be discussed in a later section.

Experiment II. 3. Analysis of non-inhibited shoots and new tubers produced by 1970 parent tubers.

As Table 5 part c indicates, in the sampling of non-inhibited shoots produced from 1970 tubers, it was necessary to combine leaf and stem tissues as the quantity of biomass produced was low. Hence, it is difficult to relate the amino-N concentration found in these shoots with the values obtained in other shoots under any nutrient regime (Table 5). However, concentrations found in root and tuber tissues are similar to those in plants grown on a high ammonium nutrition.

Analysis of amide-N in non-inhibited shoots and tubers growing from 1970 tubers shows relatively low levels. New tubers had only 60%

(3.64 mg amide-N/g dry weight) of the control tuber concentrations and stem-leaf samples had only 35-55% (4.53 mg amide-N/g dry weight) of control stem-leaf values. If should be noted that these plants did not receive an external nitrogen source and had available for growth only what was originally in the parent tuber. These tissues were one of the few cases in which amide-N levels were found to be the same as or lower than the amino acid concentrations.

DISCUSSION AND CONCLUSIONS

One of the most interesting observations was that nitrate fertilization causes inhibited plants to grow immediately, but ammonium fertilization does not, even though it was apparent from amino-N data that the ammonium is being absorbed. The nitrate treated plants show very rapid leaf development while the response to ammonium was less apparent. Control of leaf growth may be the key as without leaf development, aerial plant growth is retarded.

As the primary growth response occurred with an application of an external nitrate-N source, the analysis of the basic soluble complement of nitrogen compounds was an obvious starting point for investigation. Further, as the literature indicated (27), the amides were the basic nitrogen storage compounds in potatoes and thus these, along with amino acids, were analyzed. The most salient feature of both the amino-N and amide-N results was that stem tissues function in the storage of large quantities of these compounds.

The question of whether the nitrate fertilization actually overcomes the shoot inhibition and allows release of "bound" nitrogen or simply masks it by dilution is of critical importance. The growth of stem tissue of nitrate treated plants is approximately three times the growth of stem tissue of control plants. Hence if the nitrate fertilization was in reality only a masking due to dilution of amino and amide levels by growth, then an equivalent dilution of the control-stem values shown in Figure 11 should produce the same or lower curve than shown for nitrate-stem tissue (Table 6). It is apparent that amino and amide-N

Table 6. Comparison of spectra of ninhydrin-reacting compounds in the ethanol-soluble stem fraction of control and nitrate fertilized plants. Data presented for adjusted control was derived by accounting for greater growth of nitrate vs. control treated plants.

	RELATIVE ABSORBANCE AT SPECIFIC WAVELENGTHS (mμ)													
	600	580	560	540	520	500	480	460	440	420	400	380	360	340
Control	22	29	29	26	22	19	17	16	16	29	35	24	23	28
Adjusted Control	7	10	10	9	7	6	6	5	5	10	12	8	8	9
Nitrate	11	13	15	13	11	10	9	8	9	15	18	13	13	14

reserves in stem tissue of nitrate treated plants are not being metabolized; in fact, it appears that some of the nitrate is being reduced to amino and amide-N and stored in stem tissue. A more definitive test to show this conclusively would require growing plants with various concentrations of nitrate to determine if there is a critical level which will promote amino and amide-N utilization, as well as growth.

Physiological degeneration or senescence is another prominent feature of shoot inhibition. It is apparent that metabolites necessary for growth are released slowly as the tissue ages, and more aerial plant growth is supported. The intermediate growth response of inhibited plants to ammonium (and perhaps urea) was another interesting result. Plants absorbed ammonium as evidenced by the change in morphological appearance and the substantial increase in amino-N and amide-N concentrations in the tissues. The curious feature was that they absorbed ammonium and detoxified it but oculd not utilize it. A similar response to ammonium fertilization has also been shown with apple trees (12). It appears that this stored material does eventually become available for growth as was shown with ammonium fertilized plants in Figure 5. Thus ammonium treated plants respond similarly to control plants, except that during the period when shoots are inhibited, they take up ammonium and store it as amino-N and amide-N. The fact that ammonium fertilized plants showed an increase in amino and amide-N content and remained inhibited was an indication that this part of the nitrogen metabolism may be the site of inhibition.

The ultimate effect on inhibited shoots of injuring the tissue is the release of material necessary for growth. This may be the case with high gravity treatments. The growth of the excised shoots in liquid culture may be another example. It is quite possible that the mechanical act of cutting the stem when the shoot was separated from the parent tuber was sufficient wounding to permit growth. This injury combined with nitrogen-starved growing conditions, which might have caused physiological senescence, made mobilization of stem amino-N compounds possible. The low stem amino-N levels and the expansion of leaves (Table 3) are supportative evidence for this premise, as is the vigorous growth of shoots attached to rotting tubers.

The location of the controlling mechanism for inhibition is still unknown, but it is evident that stem tissues (including the tuber) are the most prominent storage areas for metabolic constituents. These tissues apparently store these compounds, but do not release them for growth except during senescence.

The results of the absorption spectra data (Figure 11) support the conclusion that stem tissues are the main storage areas. However, they also show that stems are storing compounds other than (or certainly in higher levels than) those found in leaf and root. It is very interesting that the most actively growing tissues contain low amounts of the storage compounds. It is difficult to say which particular compound is contributing most to the absorption spectra. Pate (28) stated that, "Certain compounds, for example asparagine, homoserine and glutamic acid, are mostly incorporated into soluble reserves and do not become generally

available to growth until the tissues that store them have become senescent". Unfortunately, he offers no data or more specific information to support his statement. However, it is unlikely that asparagine is acting in this way in potato tissues since it appears to be present in the highly active tissues, but not present in significantly large quantities in the storage tissues. Homoserine would not contribute to the amide-N component and is probably not of major concern. Glutamic acid and its amino acid amide are known to be major constituents of amino and amide-N fraction of potatoes. Further, both of these compounds would absorb in the peak areas shown in stem and tuber tissue. Thus, it is possible that the primary nitrogen storage component in inhibited plants is glutamic acid and/or glutamine. As glutamine will contribute to both amino and amide-N values, it is the more likely possibility.

There is also some reference in the literature to glutamine, in the form of glutamic acid polymers as possible storage compounds (38), e.g. glutathione (gamma-glutamylcysteinylglycine) is a classic example of a universally distributed glutamyl peptide. It is conceivable that nitrogen could be stored as one of these low molecular weight polyglutamyl peptides.

The two different mechanisms that may be involved in limiting the availability of amino and amide-N are: (1) metabolic inhibition, or (2) compartmentalization. For example, glutaminase activity might be inhibited or the enzyme could be contained in a membrane isolating it from its substrate. The result of either mechanism would be an

accumulation of glutamine since the amide group could not be liberated. Microsome coats have been shown to contain enzymes which do not become available until the protein membrane ruptures (40). Thus, during injury or senescence, the enzymes would be freed.

The obvious future course for this research is chromatographic analysis of the actual amino acid and amide fractions to determine which compounds are contributing most heavily to the observed accumulation. When this is done, more specific inhibition mechanisms could be proposed.

It is extremely interesting that the other Solanum species tested did not show the shoot inhibition response. It is conceivable than man could have selected for the shoot inhibition phenomenon in S. tuberosum by selecting for tubers with higher nitrogen content. The fascinating opportunity thus exists to determine the site of genetic control of this observed shoot inhibition.

LITERATURE CITED

1. Baker, Dennis A. 1969. Transport pathways in sprouting tubers of the potato. J. Exp. Bot. 20(63):336-340.
2. Beevers, Leonard and R. H. Hageman. 1969. Nitrate reduction in higher plants. Ann. Rev. Plant Physiol. 20:495-522.
3. Bell, Pamela M. 1963. A critical study of methods for the determination of nonprotein nitrogen. Anal. Biochem. 5:443-451.
4. Chin, Wei-Tsung and Wybe Kroontje. 1963. Urea hydrolysis and subsequent loss of ammonia. Soil Sci. Am. Proc. 27(3):316-318.
5. Davies, David D. 1968. The metabolism of amino acids in plants. In: Recent Aspects of Nitrogen Metabolism in Plants. Ed. E. J. Hewitt and C. V. Cutting. Academic Press, New York.
6. Dinkel, D. H. 1963. Light induced inhibition of potato tuber sprouting. Science 141:1047-1048.
7. Dinkel, D. H. 1968. Nitrogen metabolism of potato tubers: Assimilation of stored tuber reserves in the light and dark. Am. Soc. Hort. Sci. 93:476-484.
8. Doby, Géza. 1965. Plant biochemistry. Interscience Publishers (a division of John Wiley and Sons, Ltd) New York. pp. 768.
9. Folkes, B. F. 1959. The position of amino acids in the assimilation of nitrogen and the synthesis of proteins in plants. In: Symposia of the Society for Experimental Biology. XIII. Utilization of Nitrogen and its Compounds by Plants. Academic Press, New York.

10. Greidanus, Ted, L. A. Peterson, L. E. Schrader, and M. N. Dana. 1972. Essentiality of ammonium for cranberry nutrition. J. Amer. Soc. Hort. Sci. 97(2):272-277.

11. Hewitt, E. J., D. P. Hucklesby and G. F. Betts. 1968. Nitrite and hydroxylamine in organic nitrogen metabolism with reference to higher plants. In: Recent Aspects of Nitrogen Metabolism in Plants. Ed. E. J. Hewitt and C. V. Cutting. Academic Press, New York.

12. Hill-Cottingham, D. G. 1968. The effect of climate and time of application of fertilizers on the development and crop performance of fruit trees. In: Recent Aspects of Nitrogen Metabolism in Plants. Ed. E. J. Hewitt and C. V. Cutting. Academic Press, New York.

13. Hoagland, D. R. and D. I. Arnon. 1939. The water culture method of growing plants without soil. Calif. Agr. Exp. Sta. Cir. 347.

14. Humphries, E. C. and S. A. W. French. 1961. Effect of nitrogen supply on the response of Majestic potato to gibberellic acid. Ann. Appl. Biol. 49:331-339.

15. Ivanko, S. and J. Ingversen. 1971. Investigation on the assimilation of nitrogen by Maize roots and the transport of some major nitrogen compounds by xylem sap. III. Transport of nitrogen compounds by xylem sap. Physiol. Plant. 24:355-362.

16. Kawakami, Kojiro. 1952. Physiological aspects of potato seed tubers. The Mem. Hyogo Agr. Coll. 2:1-114.

17. Kawakami, K. 1962. The physiological degeneration of potato seed tubers and its control. Eur. Potato J. 5(1):40-49.

18. Keeney, D. R. and L. M. Walsh. 1972. Available nitrogen in rural ecosystems: Sources and fate. HortScience 7(3):219-223.

19. Kliewer, W. Mark. 1964. Influence of environment on metabolism of organic acids and carbohydrates in Vitis vinifera. I. Temperature. Plant Physiol. 39:869-880.

20. Kocher, Federico and Jorge Valenzuela. 1971. Nitrogen constituents in tissues of growing and dormant lowbush blueberry plants. HortScience 6(4):411-413.

21. Laughlin, Winston, M. 1971. Response of potato to urea and other nitrogen sources. Research report 71-1, University of Alaska, Institute of Agricultural Sciences, Fairbanks, Alaska.

22. Lewis, L. N., N. E. Tolbert and A. L. Kenworthy. 1963. Influence of mineral nutrition on the amino acid composition of bartlett pear trees. Proc. Am. Soc. Hort. Sci. 83:185-192.

23. Lowry, O. H., N. J. Rosebrough, A. L. Farr and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193:265-275.

24. Lyon, T. Lyttleton and Harry O. Buckman. 1949. The nature and property of soils. Fourth Edition. Macmillian Co. New York. 499p.

25. Malavalta, E. 1954. Studies on the nitrogenous nutrition of rice. Plant Physiol. 29:98-101.

26. McKee, H. S. 1962. Nitrogen Metabolism in Plants. Clarendon Press, Oxford. 728p.

27. Neuberger, A. and F. Sanger. 1942. Nitrogen of the potato. Biochem. J. 36:662-671.

28. Pate, J. S. 1969. Physiological aspects of inorganic and intermediate nitrogen metabolism (with special reference to the legume, Pisum arvense L.). In: Recent Aspects of Nitrogen Metabolism in Plants. Ed. E. J. Hewitt and C. V. Cutting. Academic Press, New York.

29. Rico, M. 1970. Influencia del tamamo del tuberculo sembrado en la produccion en Solanum tuberosum. (Influence of the size of the tuber sown on the production of Solanum tuberosum.) Bol. Inst. Nac. Invest. Agron. 27(57):269-277. (Abstract only.)

30. Simmonds, N. W. 1965. Attempted graft transmission of potato-tuber dormancy. Eur. Potato J. 8(4):197-199.

31. Sims, A. P., B. F. Folkes and (in part) A. H. Bussey. 1969. Mechanisms involved in the regulation of nitrogen assimilation in micro-organisms and plants. In: Recent Aspects of Nitrogen Metabolism in Plants. Ed. E. J. Hewitt and C. V. Cutting. Academic Press. New York.

32. Slack, E. B. 1949. Amino acid composition of the protein and nonprotein nitrogenous substances in the potato. Abs. Communs. 1st Intern. Congr. Biochem. pp. 98-100.

33. Smith, Ora. 1968. Potatoes: Production, storing and processing. The Avi Publishing Co., Inc. Westport, Connecticut. 642p.

34. Stahl, A. L. and J. W. Shive. 1933. Studies on nitrogen absorption from culture solutions. I. Oats. Soil Sci. 35:375-380.

35. Steward, F. C. and H. E. Street. 1946. The soluble nitrogen fractions of the potato tuber; the amides. Plant Physiol. 21: 155-204.

36. Steward, F. C., J. F. Thompson, and C. E. Dent. 1949. Gamma amino butyric acid; a constituent of the potato tuber? Science 110:439-440.

37. Steward, F. C. and J. F. Thompson. 1952. Properties and physiological role of asparagine and glutamine, with a new interpretation of the structure of asparagine. Nature 169:739-742.

38. Steward, F. C. Ed. 1965. Plant Physiology: A Treatise. Volume IVA: Metabolism: Organic Nutrition and Nitrogen Metabolism. Academic Press, New York.

39. Steward, Ivan. 1962. The effect of minor element deficiencies of free amino acids in citrus leaves. Proc. Am. Soc. Hort. Sci. 81: 244-249.

40. Tolbert, N. E. 1971. Microbodies - peroxisomes and glyoxysomes. Ann. Rev. Plant Physiol. 22:45-74.

41. Varner, J. E., W. A. Bulen, Steve Vanecko, and R. C. Burrell. 1953. Determination of ammonia, amide, nitrate and nitrite nitrogen in plant extracts. Anal. Chem. 25:1528-1529.

42. Wallace, W. and J. S. Pate. 1967. Nitrate assimilation in higher plants with special reference to the Cocklebur (Xanthium pennsylvanicum Wallr.). Ann. Bot. 31(122):213-228.

43. Weast, Robert C. and Samuel M. Selby. Eds. 1967. Handbook of Chemistry and Physics. The Chemical Rubber Co., Cleveland, Ohio.

44. Weeks, W. W., and P. A. Hedin. 1966. Amino acids in male sterile and normal watermelon. Proc. Am. Soc. Hort. Sci. 88:564-567.

45. Wildon, C. E. and F. L. S. O'Rourke. 1964. The effect of arcillite in media for pot plants. Research Report, Michigan State University, Agricultural Experiment Station. East Lansing, Michigan.

46. Yemm, E. W. and E. C. Cocking. 1955. The determination of amino acids, with ninhydrin. Analyst 80:209-214.